only a small fraction of the interesting questions that can be answered by close collaboration between the experimental and theoretical sides of the chemotaxis field.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## Primer
# Microballistics in fungi and plants

Nicholas P. Money

Ballistic movements in biology are powered by muscle contraction, explosive chemical reactions, the formation and collapse of gas bubbles, merger of fluid droplets, and release of hydrostatic pressure. At the macroscopic end of this kinetic carnival we find jumping fleas, violent spider jaws, shrimp claw hammers, and squirting beetles and cucumbers. The speeds are startling, but the mechanisms seem familiar because they occur on a spatial scale that overlaps with our physical experiences. We jump, albeit more slowly than fleas, for example, and it does not seem strange that seeds will spurt from a swollen cucumber when it hits the ground. Ballistics in microscopic dimensions are very different, operating in a seemingly alien world of fluid mechanics where thin air becomes soup and gravity vanishes.

For very small projectiles, like fungal and plant spores, the only way to escape a surface is to rocket off at an incredible speed at the get go. Even with an initial burst of momentum, many spores travel no farther than a few times their own cell length before viscous drag brings them to a dead stop and they float to the ground. Mushroom spores are discharged in this fashion, using the motion of fluid drops to power their short jumps. Greater discharge distances, ranging from tens of centimeters to meters, are achieved by ejecting spores with pressurized fluid (many fungi), the use of an air gun (*Sphagnum* moss), and a unique snap-through buckling mechanism (the artillery fungus). The effect of air viscosity on motion decreases as the projectile size increases, and gravity assumes more obvious control of the flight path. We return to familiar territory with these longer trajectories whose curvature resembles the arc of a golf ball. To survey these processes, we will use the categories of short-range, intermediate-range, and long-range microballistics.

## Short-range microballistics: micrometers to millimeters

Some discharge mechanisms do little more than separate spores from the stalks on which they develop so that they can be dispersed by the surrounding airflow. A fungus called *Deightoniella*, which grows on banana leaves, makes use of explosive bubble formation, or cavitation, within its stalks to launch its spores. Each spore of this fungus sits on a thick-walled bulb at the tip of its stalk, which shrinks in dry air, exerting tension on the cell wall (Figure 1). As drying continues, the water in the cytoplasm fractures to create a vapor-filled bubble, which allows the cell wall of the bulb to pulse outward, shooting the spores at a maximum speed of 0.6 m s$^{-1}$ over a distance of 0.5 mm. The spores travel 15 times their own cell length, which is sufficient for them to escape the boundary layer of still air on the leaf surface. Cavitation relieves the negative hydrostatic pressure
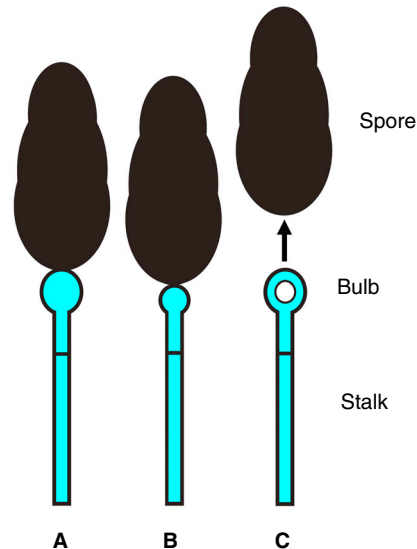


**Figure 1. The spore of the fungus *Deightoniella torulosa* is propelled from the bulbous tip of its stalk by the formation of a cavitation bubble.**
(A) The spore forms on the tip of its stalk. (B) The bulb at the tip of the stalk shrinks as water evaporates from the cytoplasm, until the cell wall resists further contraction and negative hydrostatic pressure develops within the cytoplasm of the bulb. (C) When the tension within the cytoplasm exceeds a critical threshold, a void or bubble of vapor forms in the bulb, relieving the negative pressure. Bubble formation causes the cell wall of the bulb to expand, which launches the spore.
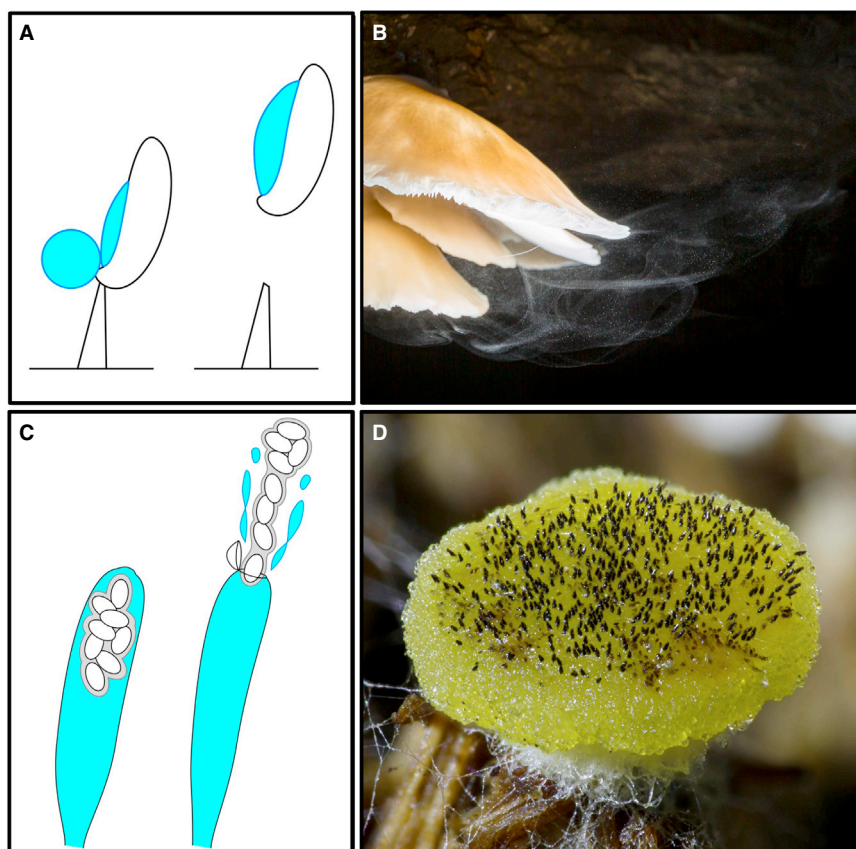
**Figure 2. Ballistospore and ascospore discharge in fungi.**
(A) Schematic of ballistospore discharge. Condensation of water on the spore surface produces two separate droplets of fluid, whose coalescence shifts the center of mass of the projectile that jumps into the air. (B) Oyster mushroom, *Pleurotus ostreatus*, smoking the air with thousands of spores discharged from its gill surfaces. (Photo: Neil Jernigan Photography.) (C) Ascospore discharge from an ascus with a lid or operculum. Osmosis pressurizes the ascus sap before the operculum flips open. (D) Fruit body or ascoma of *Ascobolus stercorarius* whose surface is dotted with unexploded asci containing pigmented ascospores. (Photo: Chris Johnson.)

that develops when water evaporates from the cytoplasm of the cell with a thick cell wall that resists collapse. The stalk cells of another fungus, *Zygophiala*, bend as they lose water and straighten with the formation of cavitation bubbles. Bending of the stalk cells occurs over a few seconds and straightening happens in microseconds.

Mushrooms and related fungi in the Basidiomycota use the condensation of water rather than evaporation to prime their mechanism of ballistospore discharge. Mannitol and inorganic ions secreted on the spore surface lower its dew point, causing water to condense as a discrete droplet on a short peg and as a separate drop of water on the adjacent surface (Figure 2A). These droplets expand as condensation

continues and snap together when they make contact, shifting the center of mass of the spore, which jumps into the air at a speed of 1 m s$^{-1}$. This process of momentum transfer works as a surface tension catapult, driven by the reduction in surface free energy, or surface tension, when the droplets coalesce. There are no comparable mechanisms elsewhere in nature. Using this catapult, as many as 30,000 spores release themselves from the gills of a mushroom every second, corresponding to billions of spores over the lifetime of the fruit body (Figure 2B).

The distance that the spores are shot within a mushroom is critical. To avoid becoming stuck on the opposing gill, they must reach a sweet spot in the middle of the air

space between gills, and freefall from the cap to be dispersed in the air currents swirling around the fruit body. Gill separation differs between species and the discharge distance appears to be fitted to each kind of mushroom by changes in the size and shape of spores that affect the size of the droplets that condense on their surface. There is a clear relationship between spore size and drop size and larger droplets pack more energy than smaller ones. Ballistospore discharge also launches the spores of jelly fungi, rusts and smuts that infect plants, and related yeasts whose spores jump farther than 1 mm without the obstacles of escaping from a mushroom cap.

**Medium-range microballistics: millimeters to centimeters**
Cavitation, which fires mould spores over short distances, also powers the discharge of fern spores from a miniature sling-shot. A rim of thick-walled cells around the ovoid sporangia of these plants serves as a spring (Figure 3). As water evaporates from these cells, the band peels back on itself like a stretched accordion, and the sporangium gapes open forming a cup that cradles the spores. As drying continues, water tension increases until cavitation bubbles erupt inside the cells, and the band springs forward expelling the spores at a speed of 10 m s$^{-1}$ in 30 μs. This is a perfect example of power amplification, which belongs to the category of latch-mediated spring actuation mechanisms that drive many of the fastest movements in biology.

Pollen catapults have evolved in other plants, and include the explosive flowers of the white mulberry tree and the bunchberry dogwood. The turgid filaments that support the pollen-filled anthers of the mulberry become bent as the flower bud develops, storing elastic energy. They are held in place by tiny threads. When the mature flower dries, these threads break and each filament springs outwards, throwing the pollen in a circular path like the arm of a cricket bowler. This hurls the pollen grains at an estimated speed of 170 m s$^{-1}$ as far as 6.6 cm. If this value for the speed is accurate, the mulberry flower ranks as the fastest known natural microballistic

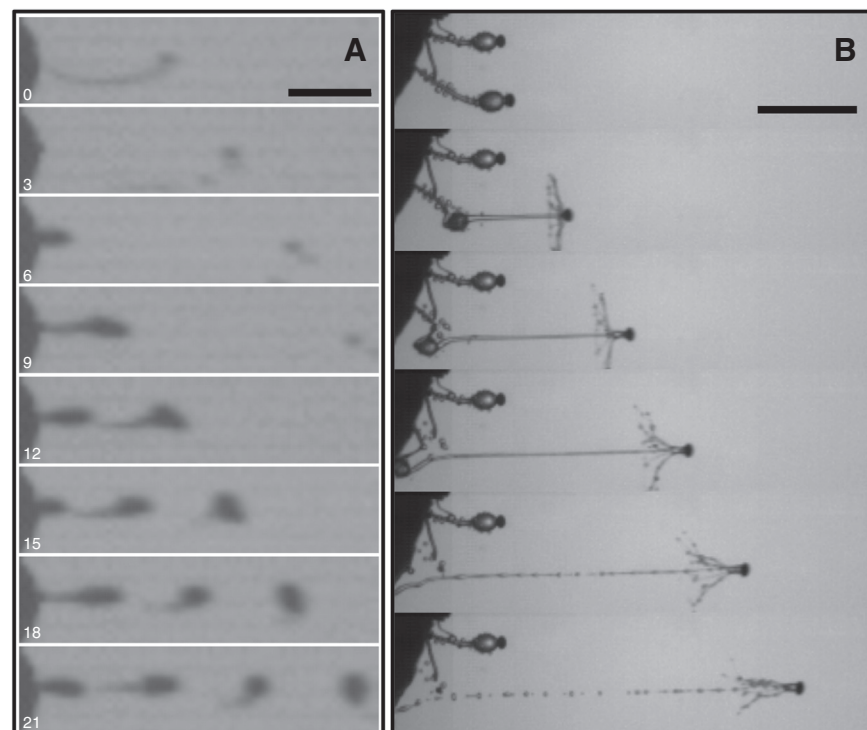**Figure 3. Cluster of discharged fern sporangia highlighted by fluorescence.**
After peeling back as it dries, the rim of thick-walled cells, which is called the annulus, springs forward expelling the spores. Two dark brown spores in this image have stuck to the sporangia. (Photo: Frank Fox/Science Photo Library.)

device. The filaments that support the pollen-filled anthers of the bunchberry dogwood are folded inside the flowers against the surrounding petals which act as latches. When the petals open, the filaments flex apart, flinging pollen grains 2.5 cm into the air at a more modest speed of 7 m s$^{-1}$. The motion of the dogwood anther is like the arm of a baseball pitcher.

The capsules of another plant, *Sphagnum* moss, shrink as they are warmed by sunlight, compressing and pressurizing the air within an impermeable chamber below the spores by up to 0.5 MPa or 4.9 atmospheres. This pressure is relieved when the lid of the capsule opens, spraying the spores at a speed of 30 m s$^{-1}$ up to 20 cm above the plant.

Pressurized fluid rather than gas propels the spores of ascomycete fungi at comparable speeds over similar distances. The devices that discharge ascospores are called asci. Sap within the asci is pressurized by osmosis and the spores are expelled when the ascus tips open via simple slits, hinged lids, reinforced rings or unfolding sleeves (Figure 2C,D). The rings and sleeves operate as valves, conserving ascus pressure as the spores move into the tip and are expelled one at a time. Ascospore shape and size vary

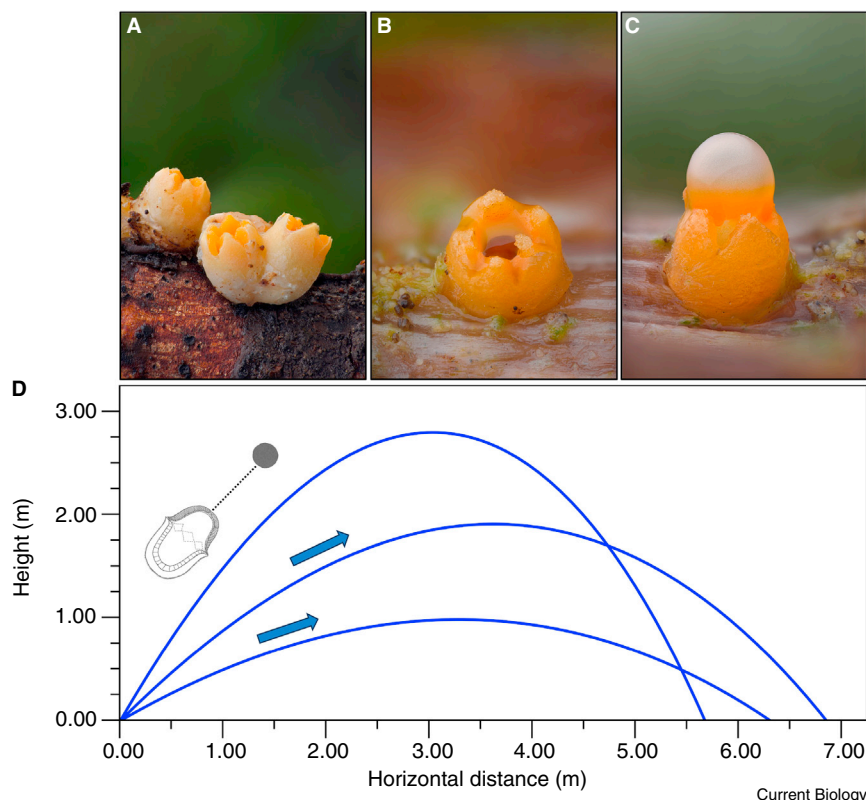a great deal and the spores of some species are tethered to one another

creating large projectiles with sufficient momentum to carry the spores over many centimeters.

Mannitol and other sugar alcohols contribute to the osmotic gradient that generates hydrostatic pressure or turgor within the ascus. This is interesting from an evolutionary perspective because ballistospore discharge in mushrooms may have evolved from some type of ascus, with the use of mannitol to increase the osmotic pressure of the ascus sap and to lower the vapor pressure of the droplets that condense on the ballistospore surface (Figure 2A,B).

High-speed video clips of ascus discharge recorded at 1 million frames per second have clocked a maximum launch speed of 32 m s$^{-1}$ from a fungus called *Neurospora tetrasperma* (Figure 4A). The spores reach this speed as soon as they slip from the tip of the ascus, which takes a few microseconds and involves tremendous acceleration. *Hydra* nematocysts are even faster: their stylets are fired at a speed



**Figure 4. High-speed spore discharge.**
(A) The four ascospores of *Neurospora tetrasperma* are discharged at a velocity of up to 32 m s$^{-1}$ and rotate during flight. Images stamped with time in μs from video recorded at 1 million frames per second. Scale bar = 20 μm. (B) Sporangium of *Pilobolus kleinii* is squirted from its stalk at a top speed of 13 m s$^{-1}$. The stalk collapses as it recoils with the expulsion of fluid. Images separated by 200 μs from a video recorded at 50,000 fps. Scale bar = 2 mm.

**Figure 5. Sporangial discharge in the artillery fungus, *Sphaerobolus stellatus*.**
(A) Trio of fruit bodies that opened very recently showing a toothed or star shape produced by the fracture of the tissue layers surrounding the upper surface of the sporangium that is visible in (B). (C) Discharged fruit body with everted inner cup that is connected to the outer cup on the tips of the triangular teeth. (D) Modeled trajectories of sporangia launched at 30°, 45°, and 60° using a simple model based on Stokes' law for viscous drag on the projectile. The maximum measured discharge distance is 6 m. (Photos: Timothy Boomer.)

interpolation models, in which the motion of the spore is calculated in an iterative fashion as it moves through the air, fail to predict actual speeds from measured discharge distances and vice versa.

**Long-range microballistics: meters**
Turgor pressure powers the spore discharge mechanisms with the longest ranges in a pair of fungi that grow on herbivore dung. *Pilobolus*, the hat thrower, uses the better known of these processes. This works as a miniature squirt gun that shoots a black sporangium at a speed of 9 m s$^{-1}$ from a centimeter-tall stalk over a 2 m parabolic trajectory (Figure 4B). The ejection of a long jet of fluid from the stalk has little effect on the range of the sporangium because it trails behind the sporangium rather than continuing to push. Pressure in the transparent fluid before discharge is around 0.5 MPa, or 5 atmospheres, and is generated by inorganic ions mixed with low concentrations of sugar alcohols. The turgor pressures involved in spore discharge are no higher than the pressures measured from the vegetative hyphae that form the feeding colonies or mycelia of fungi. So, rather than harnessing especially high levels of pressure, the remarkable feature of the turgor-driven ballistic mechanisms is the way that different species have customized their launch devices using constitutive levels of cellular pressures.

The second of the dung fungi with a long-range mechanism of spore discharge is the artillery fungus, *Sphaerobolus*. This offers an unusual example of a turgor-driven apparatus that does not involve any expulsion of fluid (Figure 5). The 2 mm-diameter fruit body of the artillery fungus is structured as a rigid outer cup that supports an interior elastic cup that cradles the sporangium. Turgor pressure within the inner cup compresses the ends of the cells beneath the sporangium and stretches the cells on the other side facing the outer cup. This stress gradient is reversed when the cup flips outward, flinging the sporangium 6 m onto vegetation surrounding the animal dung. Herbivores browsing on these plants beyond the zone of repugnance disperse the spores after passage

of 19 m s$^{-1}$, with an estimated acceleration of 5 million *g*, and stop dead within a microsecond. Even so, ascospore discharge is certainly among the swiftest movements in biology. Spore rotation is another amazing metric. The ellipsoidal spores of *Neurospora* spin end over end during flight at a rate of 40,000 revolutions per second, or 2 million revolutions per minute. Although the difference in size and duration renders the following physical comparison frivolous, these spores spin faster than neutron stars.

Turgor pressure powers a different kind of medium-range discharge mechanism in the 'zombie-fly fungus', *Entomophthora*. This fungus induces a climbing behavior in infected insects, positioning itself for the wind dispersal of its spores. After the fly dies, the fungus bursts through the intersegmental membranes of its

abdomen, forming thousands of 'soft water cannons' that discharge spores from their tips when they burst. The release of the spore is associated with the atomization of a small quantity of pressurized liquid and retention of most of the propellant inside the collapsing stalk. This differs from the discharge of ascospores along with almost all of the surrounding ascus sap.

The discharge distance of spores depends on their size and speed, and air viscosity. Using Stokes' law to calculate the effect of viscous drag offers an excellent fit to the experimental data on spore discharge. Stokes' law provides a measure of the frictional force acting on particles as they move through a liquid or air. Its use assumes that the Reynolds number (*Re*), which is a measure of the ratio of inertial to viscous forces, remains relatively constant for a spore from its launch to deposition. More complex

through their digestive systems. The term snap-through buckling captures the essence of this discharge process, in which the eversion of the elastic membrane releases energy stored in its pressurized cells. The Venus fly trap and jumping popper toy operate on the same elastic snap-through principle.

Considering the energetic cost of the buckling mechanism, we find that sporangial discharge by the artillery fungus consumes significantly more energy than ballistospore discharge to release an equivalent number of spores: 2 billion ballistospores propel themselves from the gills of a single mushroom with a combined kinetic energy ($\frac{1}{2}mv^2$) of $7 \times 10^{-5}$ J (for an estimated per spore kinetic energy of $3.5 \times 10^{-14}$ J); 200 fruit bodies of the artillery fungus release 2 billion spores with a kinetic energy of $5.6 \times 10^{-3}$ J (for an estimated per sporangium kinetic energy of $2.8 \times 10^{-5}$ J). On the basis of energy per unit mass, the artillery fungus costs 40 J kg$^{-1}$, compared with 0.1 to 2.0 J kg$^{-1}$ for the discharge of the largest and smallest ballistospores. The apparent extravagance of the *Sphaerobolus* mortar is probably explained by the relatively low wastage of spores carried in its sporangia compared with the losses of wind-dispersed spores from gilled mushrooms. If the average spore of the artillery fungus has a 20- to 400-fold greater probability of surviving and forming a new mycelium than a spore that forms on a mushroom gill, the reproductive costs of the different mechanisms will be the same.

The launch of the artillery fungus is at the upper end of the arbitrary category of microballistics, where the projectiles become visible without a microscope. The discharge of even larger projectiles takes us to the bird's nest fungi, whose 2 mm-diameter sporangia are splashed from their flute-shaped fruit bodies, and a plethora of plant species whose seeds are thrown from twisting and fracturing capsules. Air viscosity loses its dominance over the flight of these projectiles, becoming subordinate to the effects of gravity, and the aerodynamics merge with the realms of motion similar to our own.

The ballistic mechanisms that have evolved among fungi and plants are united by the movement of water, either by osmosis to pressurize the cytoplasm, condensation to create mobile droplets, evaporation to deform elastic cell walls, or the impact of raindrops. In the absence of musculature, these hydraulic processes are the only way to power the propulsion of spores, pollen, and seeds through air.

## DECLARATION OF INTERESTS

The author declares no competing interests.

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## Primer

# Biophysics of protist behaviour

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Protists, an umbrella term first coined by Ernst Haeckel in 1866, are a vast collection of (primarily unicellular) eukaryotes that are "neither animals nor plants". This basic definition by exclusion has been exercised for centuries, even though recent advances have led to more rigorous taxonomic assignment of various protist groups. Pioneering comparative phylogenetic approaches have been applied to these organisms to reconstruct the deep branches of the eukaryotic tree, revealing essential clues about early eukaryotic evolution. Protists, including amoebae, flagellates, ciliates, and algae, are also vital constituents of global ecosystems, where they appear at the base of food chains, control the relative abundance of other microbes, and participate in global biogeochemical recycling. Due to their typically small size and lack of nervous systems, protists are often associated with the unfortunate label 'primitive'. Yet they exhibit remarkable behavioural sophistication and are able to feed, predate, navigate and interact with their surroundings. Unlike macroscopic animals, many protists reside in a non-intuitive physical regime where viscous forces dominate over inertia, and where they use diverse propulsion and navigation strategies. Interdisciplinary research into these cell-scale phenomena, characterised by a complex interplay of physical forces and mechanical constraints, has significantly advanced the emerging fields of active matter, microhydrodynamics, and non-equilibrium statistical physics. This primer discusses the biophysics of protist behaviour, with a focus on locomotion and feeding. I will highlight the most extensively studied principles and describe some more esoteric behaviours that have not yet been systematically explored.

## Species diversity

Protists represent the majority of the Earth's eukaryotic diversity, with a